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Project Page Link: <https://review.jove.com/account/file-uploader?src=21452223>

Title: Stereotaxic Surgery in Infant Mice and Postoperative Care

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

If **Yes**, please provide the **full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author willing to participate.

Jingheng Zhou, Contract Biologist, Neurobehavioral Circuits Group, NIEHS

Julieta Lischinsky, Stadtman Investigator, Neurobehavioral Circuits Group, NIEHS

Current Protocol Length

Number of Steps: 23

Number of Shots: 49

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

1.1. **Julieta Lischinsky:**

Our research focuses on understanding the developmental, cellular, and circuit mechanisms for the establishment of social behaviors.

~~Describe the research contexts or systems in which this protocol can be applied.~~

1.2. **Julieta Lischinsky:**

This approach is very flexible, as any AAV construct can be injected to study the infant brain.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

1.3. **Jingheng Zhou:**

This protocol enables studying cells of interest and their anatomical circuitry, neuronal dynamics, and cellular role across postnatal development.

~~Describe the most important consideration or challenge when performing this protocol.~~

1.4. **Jingheng Zhou:**

Key considerations in this protocol include adjusting adult coordinates for the infant brain and ensuring pup acceptance by the dam post-surgery.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Jingheng Zhou/Julieta Lischinsky:**

Future studies can explore how early life adversity and environmental toxicants disrupt neuronal mechanisms underlying social behavior during postnatal development.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What value do you expect this video article to have for other researchers working in your field?

- 1.3. **Enter author name, Enter author title:** (authors will present their testimonial statements live)
- 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

How does the video format of your article help you to share your work with the broader scientific community?

- 1.4. **Enter author name, Enter author title:** (authors will present their testimonial statements live)
- 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Testimonial added by the Author

Julieta Lischinsky:

This protocol allows to perform stereotaxic surgery in infant mice and postoperative care.

Videographer's NOTE: The authors did the testimonials, but I'm not really sure they answered the question.

Ethics Title Card

This research has been approved by the National Institute of Environmental Health Sciences
Animal Care and Use Committee at the National Institute of Environmental Health Sciences

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Surgical Preparation of Infant Mice

Demonstrator: Jingheng Zhou

- 2.1. To begin, prepare the stereotaxic apparatus with stereotaxic accessories for infant mice [1], including a pair of non-rupture ear bars [2], a modified gas anesthesia head holder [3], and a palate bar for postnatal day 12 infants [4].
 - 2.1.1. WIDE: Talent arranging the stereotaxic apparatus and placing all required accessories on the workspace.
 - 2.1.2. Close-up of the pair of non-rupture ear bars.
 - 2.1.3. Close-up of the modified gas anesthesia head holder.
 - 2.1.4. Close-up of the palate bar.
- 2.2. Mount the anesthetized mouse on the modified stereotaxic apparatus using infant accessories [1-TXT]. Place non-puncture ear bars on both sides of the head and align them equally with the stereotaxic frame ruler [2]. Set the stereotaxic ruler to 5 millimeters for postnatal day 11 infants [3].
 - 2.2.1. Talent positioning the mouse on the stereotaxic apparatus. **TXT: Anesthesia: 3% isoflurane**
 - 2.2.2. Close-up of ear bars being placed on both sides of the head and aligned symmetrically with the stereotaxic frame ruler.
 - 2.2.3. Close-up of stereotaxic ruler set to 5 millimeters.
- 2.3. Using a 1 milliliter syringe with a 27 gauge needle, inject 5 milligram per kilogram Meloxicam via the subcutaneous intrascapular route [1].
 - 2.3.1. Close-up of drug being injected under the skin between the shoulders.
- 2.4. Dilute 0.5 percent bupivacaine with saline at a 1 to 10 ratio to obtain a 0.5 milligram per milliliter solution [1]. Inject the solution subcutaneously under the skin of the head [2].
 - 2.4.1. Talent adding bupivacaine to saline.
 - 2.4.2. Talent injecting solution under the scalp.
- 2.5. Using small scissors, perform a 1.5 centimeter incision along the midline of the head [1].
 - 2.5.1. Close-up of incision being made along the scalp.

- 2.6. Using a sterile cotton swab with betadine, remove the soft tissue from the skull surface [1].

2.6.1. Talent cleaning the skull surface with a cotton swab.

3. Stereotaxic Positioning and Craniotomy

- 3.1. Place a pulled glass capillary approximately 0.8 millimeters in length into a glass pipette holder [1]. Mount the holder onto the vertical stereotaxic arm [2].

3.1.1. Talent inserting the capillary into the holder.

3.1.2. Talent mounting the holder onto the stereotaxic arm.

- 3.2. Under a dissecting microscope, move the capillary to the rostral confluence of the dorsal sinuses or RCS (*R-C-S*) point [1].

3.2.1. ~~SCOPE:~~ Close-up shot of capillary positioned at the RCS point. **NOTE: This scope shot has been changed to a close-up shot.**

- 3.3. Under 2 times magnification, zero the X, Y, and Z coordinates when the capillary touches the skull [1]. Move the capillary to lambda at Y equals minus 7.30 and lower it to touch the skull [2]. Adjust the anterior-posterior angle in the stereotaxic rig until the difference in the Z values between the RCS and lambda is less than 0.02 millimeters [3].

3.3.1. Shot of coordinate values being zeroed.

3.3.2. ~~SCOPE:~~ Close-up shot of the capillary being moved to the lambda and lowered to touch the skull. **NOTE: This scope shot has been changed to a close-up shot.**

3.3.3. Talent adjusting the stereotaxic rig.

- 3.4. After moving the capillary to the RCS point, zero X and Y coordinates [1]. To level the head using the posterodorsal medial amygdala or MeA (*M-E-A*) coordinates, move the capillary to X equals positive 2.50 millimeters and Y equals negative 4.20 millimeters [2] and zero Z as the capillary slightly touches the skull [3]. Move the capillary to X equals negative 2.50 millimeters and Y equals negative 4.20 millimeters in the other hemisphere and lower the capillary to touch the skull [4]. Adjust medial-lateral axis in the stereotaxic rig until the difference in the Z values between the left and right hemispheres is less than 0.03 millimeters [5].

3.4.1. Shot of X and Y values being reset to zero.

3.4.2. ~~SCOPE:~~ Close-up shot of the capillary being moved along the X-axis to +2.50 mm and then along the Y-axis to -4.20 mm on the coordinate scale. **NOTE: This scope shot has been changed to a close-up shot.**

3.4.3. ~~SCOPE:~~ Close-up shot of the capillary touching the skull surface as the Z is zeroed. **NOTE: This scope shot has been changed to a close-up shot.**

3.4.4. ~~SCOPE:~~ Close-up shot of the capillary traversing across the skull midline to the other hemisphere coordinates and being lowered carefully to contact the skull. **NOTE: This scope shot has been changed to a close-up shot.**

3.4.5. Talent adjusting the stereotaxic rig.

3.5. After removing the capillary holder, mount a micro drill onto the stereotaxic arm [1]. Position the drill at target coordinates and drill a 0.5 millimeter diameter craniotomy through the skull [2]. Clean the skull surface carefully [3].

3.5.1. Talent replacing capillary holder with drill setup.

3.5.2. Close-up of the drill being aligned to the target coordinates and drilling into the skull.

3.5.3. Talent cleaning skull with wipes and swabs.

4. Viral Injection

4.1. Using a microfil flexible needle, fill a new capillary with mineral oil [1]. Attach and tighten the capillary to the nanoliter injector [2].

4.1.1. Talent filling the capillary with mineral oil.

4.1.2. Talent attaching the capillary to the injector.

4.2. After removing the micro drill, mount the nanoliter injector onto the stereotaxic arm [1]. Withdraw at least double the required volume of adeno-associated virus of interest [2] at a rate of 10 nanoliters per second [3].

4.2.1. Talent replacing drill with injector setup.

4.2.2. TEXT ON A PLAIN BACKGROUND:

Virus utilized in this protocol:

AAV2-DIO-mCherry - 400 nL

BSL-1

2.5E+13 vg/mL

3 µL/tube

Stored at -80°C and transferred to ice for surgery

4.2.3. ~~SCOPE:~~ Close-up shot of the injector drawing viral solution. **NOTE: This scope shot has been changed to a close-up shot.**

4.3. As previously demonstrated, after zeroing the X and Y coordinates, move the capillary to the target coordinates [1]. Lower the capillary to the skull surface [2], then zero the Z coordinate [3].

4.3.1. Shot of capillary being moved to the target coordinates.

4.3.2. ~~SCOPE:~~ Close-up shot of the capillary being lowered until it reaches the skull surface. **NOTE: This scope shot has been changed to a close-up shot.**

4.3.3. Shot of Z coordinate being reset to zero.

4.4. Lower the capillary to the desired depth and withdraw it to create a path [1]. Inject approximately 8 nanoliters of virus to ensure the capillary is not clogged [2]. Clean the capillary if clogged using sterile saline [3].

- 4.4.1. ~~SCOPE:~~ Close-up shot of the capillary entering and exiting brain tissue. **NOTE:**
This scope shot has been changed to a close-up shot.
- 4.4.2. ~~SCOPE:~~ Close-up shot of the capillary tip positioned above a sterile surface showing a tiny droplet forming and exiting the tip. **NOTE: This scope shot has been changed to a close-up shot.**
- 4.4.3. Talent cleaning capillary tip.
- 4.5. After lowering the capillary again to the desired depth, inject the virus at a rate of 20 nanoliters per minute [1]. Wait 5 minutes before withdrawing the capillary slowly [2].
 - 4.5.1. ~~SCOPE:~~ Close-up shot of the virus being injected into the target region. **NOTE:**
This scope shot has been changed to a close-up shot.
 - 4.5.2. Talent slowly withdrawing the capillary.
- 4.6. Using a 7-0 (*seven-zero*) non-absorbable suture, suture the skin [1].
 - 4.6.1. Close-up of suturing the scalp.
- 4.7. Using sterile cotton swabs, clean the suture area with 3 percent hydrogen peroxide followed by saline [1].
 - 4.7.1. Talent cleaning sutured area with swabs.

5. Post-operative Care

- 5.1. After removing the infant from the stereotaxic frame, place it in a clean cage on a heating pad [1]. Administer sterile saline to prevent dehydration [2].
 - 5.1.1. Talent transferring infant to a clean cage.
 - 5.1.2. Talent administering saline injection.
- 5.2. Monitor the infant until it becomes mobile [1].
 - 5.2.1. Close-up of infant recovering movement.
- 5.3. ~~Then,~~ Place the dam into a clean cage for approximately 2 minutes [1].
 - 5.3.1. Talent transferring dam to a separate cage.
- 5.4. Return the infant to its original home cage with littermates [1] and wipe all infants with the nest and bedding material [2]. Return the dam to the cage [3].
 - 5.4.1. Talent placing infant with littermates.
 - 5.4.2. Talent wiping the infants with nest and bedding material.
 - 5.4.3. Talent reintroducing dam into cage.
- 5.5. Confirm that the dam grooms the surgical infant without pulling the sutures [1].
 - 5.5.1. Close-up of dam interacting with the surgical infant.

Results

6. Results

- 6.1. A 90.2 percent plus or minus 2.1 overlap between mCherry (*M-Cherry*) positive and Foxp2 (*Fox-P-2*) positive cells was observed in the medial amygdala [1]. It indicated that mCherry expression was restricted to Foxp2 positive cells in a cre-dependent manner, highlighting the target efficiency [2].
 - 6.1.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the bar and the clustered dots together.*
 - 6.1.2. LAB MEDIA: Figure 3D.
- 6.2. A total of 93.7 percent plus or minus 1.4 of mCherry positive cells were located within the medial amygdala posterodorsal region [1], indicating that viral expression was restricted to the region of interest [2].
 - 6.2.1. LAB MEDIA: Figure 3E. *Video editor: Highlight the bar and the clustered dots together.*
 - 6.2.2. LAB MEDIA: Figure 3E.

Pronunciation guides:

1. Stereotaxic
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereotaxic>
IPA: /ˌster.i.əʊˈtæks.ɪk/
Phonetic Spelling: steh·ree·oh·tak·sik
2. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>
IPA: /ˌaɪ.souˈflʊr.eɪn/
Phonetic Spelling: eye·soh·floor·ayn
3. Meloxicam
Pronunciation link: <https://www.merriam-webster.com/medical/meloxicam>
IPA: /məˈlɔːk.sɪ.kæm/
Phonetic Spelling: muh·laak·suh·kam
4. Subcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌb.kjuˈteɪ.ni.əs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs

5. Intrascapular
Pronunciation link: <https://www.howtopronounce.com/intrascapular>
IPA: /ˌɪn.trəˈskæp.jə.lə/
Phonetic Spelling: in·truh·skap·yuh·lur
6. Bupivacaine
Pronunciation link: <https://www.merriam-webster.com/medical/bupivacaine>
IPA: /ˌbjuː.pɪˈveɪ.keɪn/
Phonetic Spelling: byoo·puh·vay·kayn
7. Betadine
Pronunciation link: <https://www.merriam-webster.com/medical/Betadine>
IPA: /ˈbeɪ.təˌdaɪn/
Phonetic Spelling: bay·tuh·dyne
8. Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /ˈkæp.əˌleɪ.ri/
Phonetic Spelling: kap·uh·lair·ee
9. Rostral
Pronunciation link: <https://www.merriam-webster.com/medical/rostral>
IPA: /ˈrɑː.strəl/
Phonetic Spelling: raa·struhl
10. Confluence
Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
IPA: /ˈkɑːn.flu.əns/
Phonetic Spelling: kaan·floo·uhns
11. Magnification
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnification>
IPA: /ˌmæɡ.nə.fəˈkeɪ.jən/
Phonetic Spelling: mag·nuh·fuh·kay·shuhn
12. Amygdala
Pronunciation link: <https://www.merriam-webster.com/dictionary/amygdala>
IPA: /əˈmɪɡ.də.lə/
Phonetic Spelling: uh·mig·duh·luh
13. Craniotomy
Pronunciation link: <https://www.merriam-webster.com/medical/craniotomy>
IPA: /ˌkreɪ.niˈɑː.tə.mi/
Phonetic Spelling: kray·nee·aa·tuh·mee
14. Nanoliter
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanoliter>

IPA: /'næn.ʊʊ.li:.tə/

Phonetic Spelling: nan·oh·lee·tur

15. Adeno-associated

Pronunciation link: <https://www.howtopronounce.com/adeno-associated>

IPA: /ə,di:.noʊ ə'soʊ.si.eɪ.tɪd/

Phonetic Spelling: uh·dee·noh uh·soh·see·ay·tuhd

16. Hemisphere

Pronunciation link: <https://www.merriam-webster.com/dictionary/hemisphere>

IPA: /'hem.ə,sfɪr/

Phonetic Spelling: hem·uh·sfeer

17. Dehydration

Pronunciation link: <https://www.merriam-webster.com/dictionary/dehydration>

IPA: /,di:.haɪ'dreɪ.ʃən/

Phonetic Spelling: dee·hy·dray·shuhn

18. Medial

Pronunciation link: <https://www.merriam-webster.com/dictionary/medial>

IPA: /'mi:.di.əl/

Phonetic Spelling: mee·dee·uhl

19. Posterodorsal

Pronunciation link: <https://www.howtopronounce.com/posterodorsal>

IPA: /,pɑ:.stə.ʊʊ'dɔ:r.səl/

Phonetic Spelling: paa·stuh·roh·dor·suhl

20. Cre-dependent

Pronunciation link: <https://www.howtopronounce.com/cre-dependent>

IPA: /'kri: dɪ'pen.dənt/

Phonetic Spelling: kree dih·pen·duhnt

21. Foxp2

Pronunciation link: <https://www.howtopronounce.com/foxp2>

IPA: /'fɑ:ks pi: tu:/

Phonetic Spelling: faaks pee too

22. mCherry

Pronunciation link: <https://www.howtopronounce.com/mcherry>

IPA: /ɛm'tʃer.i/

Phonetic Spelling: em·chair·ee